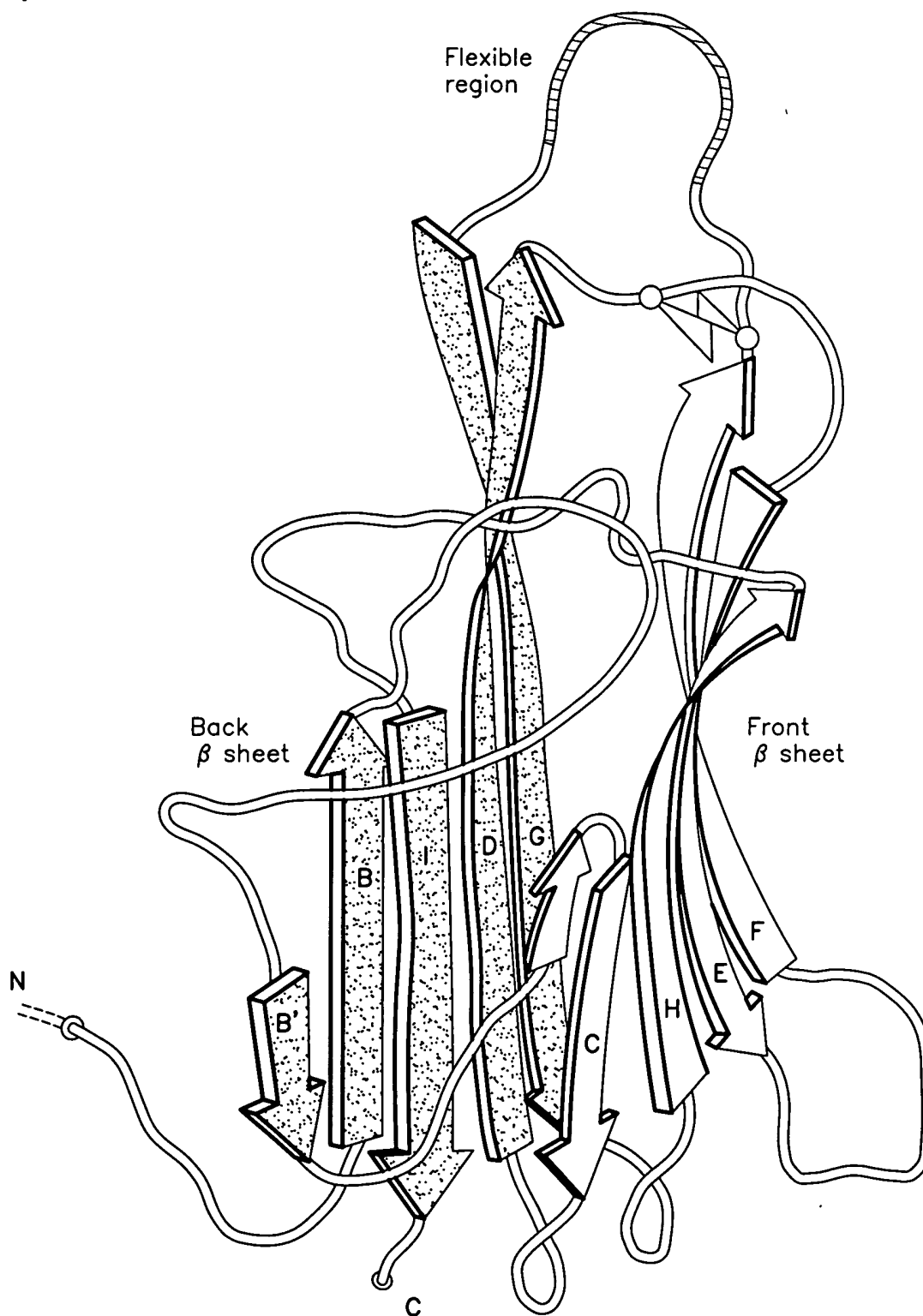




Application No. 09/060,294
Attorney Docket No. P60953US1
Replacement Sheet

FIG. 1a

Crystal Structure of TNF





Application No. 09/060,294
Attorney Docket No. P60953US1
Replacement Sheet

FIG. 1b

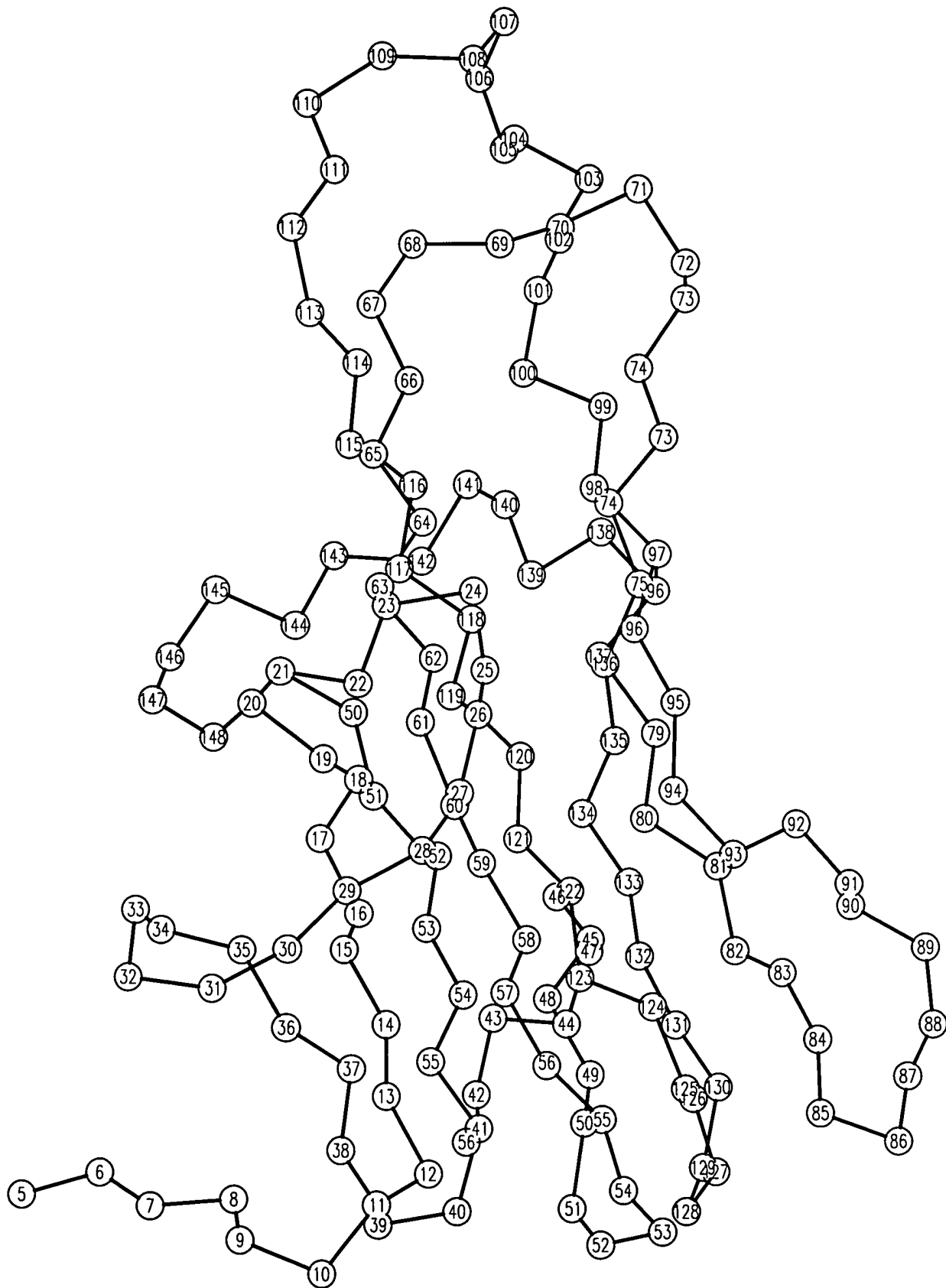




FIG. 1c

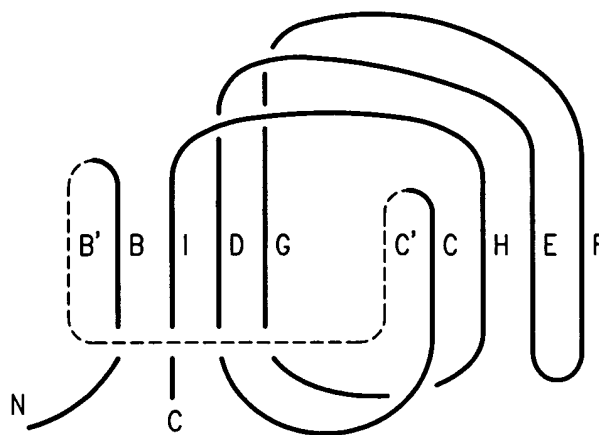


FIG. 2

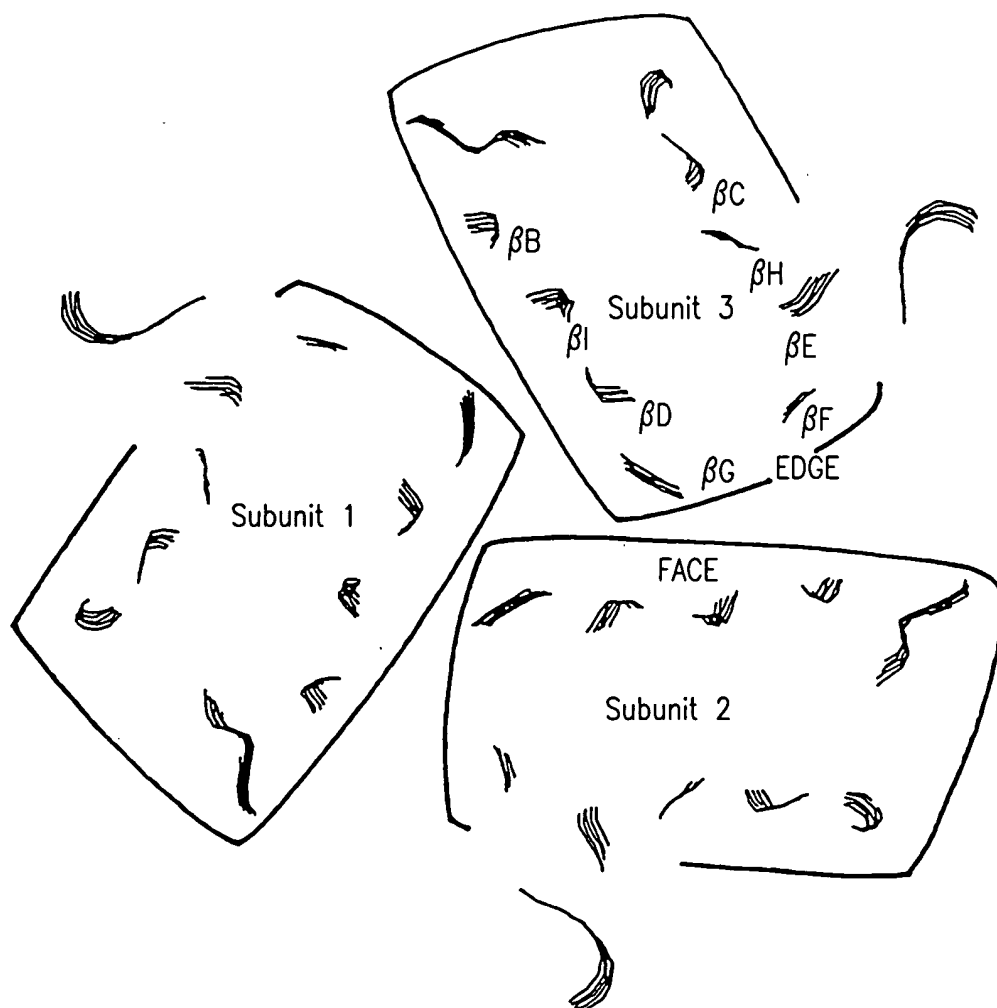
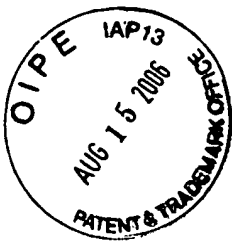




FIG. 3a

1	cacaccctga	caagctgcca	ggcaggttct	cttcctctca	catactgacc
51	cacggetcca	ccctctctcc	cctggaaagg	acaccatgag	cactgaaagc
101	atgatccggg	acgtggagct	ggccgaggag	gcgtcccca	agaagacagg
151	ggggccccag	ggctccaggc	ggtgcttgtt	cctcagcctc	ttctccttcc
201	tgatcgtggc	aggcgccacc	acgtctttct	gcctgctgca	ctttggagtg
251	atcggcccc	agaggggaaga	gtcccccagg	gacctctctc	taatcagccc
301	tctggcccag	gcagtcagat	catcttctcg	aaccccgagt	gacaagcctg
351	tagcccatgt	tgtagcaaac	cctcaagctg	aggggcagct	ccagtggctg
401	aaccgcggg	ccaatgccct	cctggccaat	ggcgtggagc	tgagagataa
451	ccagctggtg	gtgccatcag	agggcctgta	cctcatctac	tcccagggtcc
501	tcttcaaggg	ccaaggetgc	ccctccaccc	atgtgctcct	cacccacacc
551	atcagccgca	tcgccgtctc	ctaccagacc	aaggtcaacc	tcctctctgc
601	catcaagagc	ccctgccaga	gggagacccc	agagggggct	gaggccaagc
651	cctggtatga	gcccatctat	ctgggagggg	tcttcagct	ggagaagggt
701	gaccgactca	gcgctgagat	caatcggccc	gactatctcg	actttgccga
751	gtctgggcag	gtctactttg	ggatcattgc	cctgtgagga	ggacgaacat
801	ccaacettcc	caaacgcctc	ccctgcccc	atccctttat	tacccctcc
851	ttcagacacc	ctcaacctct	tctggctcaa	aaagagaatt	gggggcttag
901	ggtcggaacc	caagcttaga	actttaagca	acaagaccac	cacttcgaaa
951	cctgggattc	aggaatgtgt	ggcctgcaca	gtgaagtget	ggcaaccact
1001	aagaattcaa	actggggcct	ccagaactca	ctggggccta	cagctttgat
1051	ccctgacatc	tggaatctgg	agaccaggga	gcctttggtt	ctggccagaa
1001	tgetgcagga	cttgagaaga	cctcacctag	aaattgacac	aagtggacct
1151	taggccttcc	tctctccaga	tgtttccaga	cttccttgag	acacggagcc
1201	cagccctccc	catggagcca	gtccctctca	tttatgtttg	cacttgtgat
1251	tatttattat	ttatttatta	tttatttatt	tacagatgaa	tgtatttatt
1301	tgggagaccg	gggtatcctg	ggggacccaa	tgtaggagct	gccttggtc
1351	agacatgttt	tccgtgaaaa	cggaggctga	acaataggct	gttcccatgt
1401	agccccctgg	cctctgtgcc	ttcttttgat	tatgtttttt	aaaatattat
1451	ctgattaagt	tgtctaaaca	atgctgattt	ggtgaccaac	tgtcactcat
1501	tgetgaggcc	tctgctcccc	agggagtgtg	gtctgtaatc	ggcctactat
1551	tcagtggcga	gaaataaagg	ttgcttagga	aagaa	



Application No. 09/060,294
Attorney Docket No. P60953US1
Replacement Sheet

FIG. 3b

Amino acid sequence for human TNF α ⁸

Accession code: Swissprot P01375

-76 MSTESMIRDV ELAEEALPKK TGGPQGSRRCLFLSLFSFLI VAGATTLFCL
-26 LHFGVIGPQR EEFPRDLSLI SPLAQA
1 VRSSSRTPSD KPVAAHVANP QAEGQLQWLN RRANALLANG VELRDNQLWV
51 PSEGLYLIYS QVLFGKGQGCP STHVLLTHTI SRIAVSYQTK VNLLSAIKSP
101 CQRETPEGAE AKPWYEPIYL GGVFQLEKGD RLSAEINRPD YLDFAESGQV
151 YFGIHAL

Conflicting sequence F→S at position -14. Disulphide bond between Cys69-101. Signal anchor sequence -41 to -21 (underlined). Myristylation on Lys-58/-57.



FIG. 4a

Location of inserted epitopes

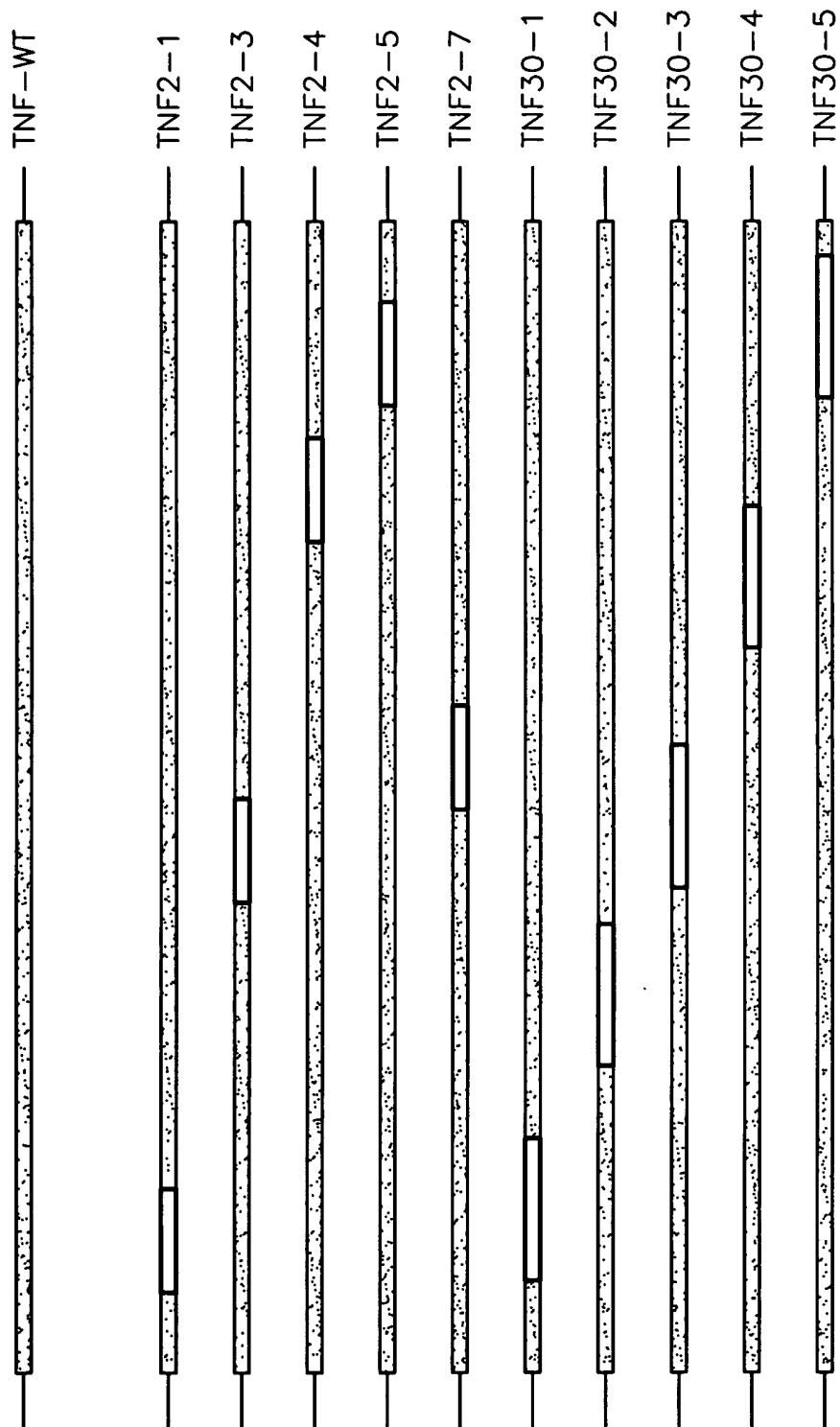




FIG. 4b

#	1	11	21	31	41	51	61	71
WT	VRSSRTPSD	KPVAHWANP	QAEQQLQWLN	RRANALLANG	VELRDNQLW	PSEGLYLYS	QVLFKGQGCP	STHVLLTHTI
2-1	-----Q	YIKANSKFIG	ITEL-----	-----	-----	-----	-----	-----
2-3	-----	-----	-----	-----	-----	-----	QYIKANSKFIGITEL	-----
2-4	-----	-----	-----	-----	-----	-----	-----	-----
2-5	-----	-----	-----	-----	-----	-----	-----	-----
2-7	-----	-----	-----	-----	-----	-----	-----	-----
30-1	-----F	NNFTVSFWLR	VPKVSASHLE	-----	-----	-----	-----	QYIKA
30-2	-----	-----	-----	-----F	NNFTVSFWLR	VPKVSASHLE	-----	-----
30-3	-----	-----	-----	-----	-----	-----	-----FNNFTVS	FWLRVPKVSA
30-4	-----	-----	-----	-----	-----	-----	-----	-----
30-5	-----	-----	-----	-----	-----	-----	-----	-----

#	81	91	101	111	121	131	141	151
WT	SRIAVSYQTK	VNLLSAIKSP	CQRETPEGAE	AKPWYEPIYL	GGVFQLEKGD	RLSAEINRPD	YLDFAESGQV	YFGIAL
2-1	-----	-----	-----	-----	-----	-----	-----	-----
2-3	-----	-----	-----	-----	-----	-----	-----	-----
2-4	-----	-----	-----	-----QYIKANS	KFIGITEL	-----	-----	-----
2-5	-----	-----	-----	-----	-----	-----QYKANSKF	IGITEL	-----
2-7	NSKFIGITEL	-----	-----	-----	-----	-----	-----	-----
30-1	-----	-----	-----	-----	-----	-----	-----	-----
30-2	-----	-----	-----	-----	-----	-----	-----	-----
30-3	SHLE	-----	-----	-----	-----	-----	-----	-----
30-4	-----	-----	-----FNNFT	VSFWRVPKV	SASHLE	-----	-----	-----
30-5	-----	-----	-----	-----	-----	-----FNNFTVSFW	LRVPKVSASH	LE



FIG. 5a

TNF α analogs with the P30 epitope inserted

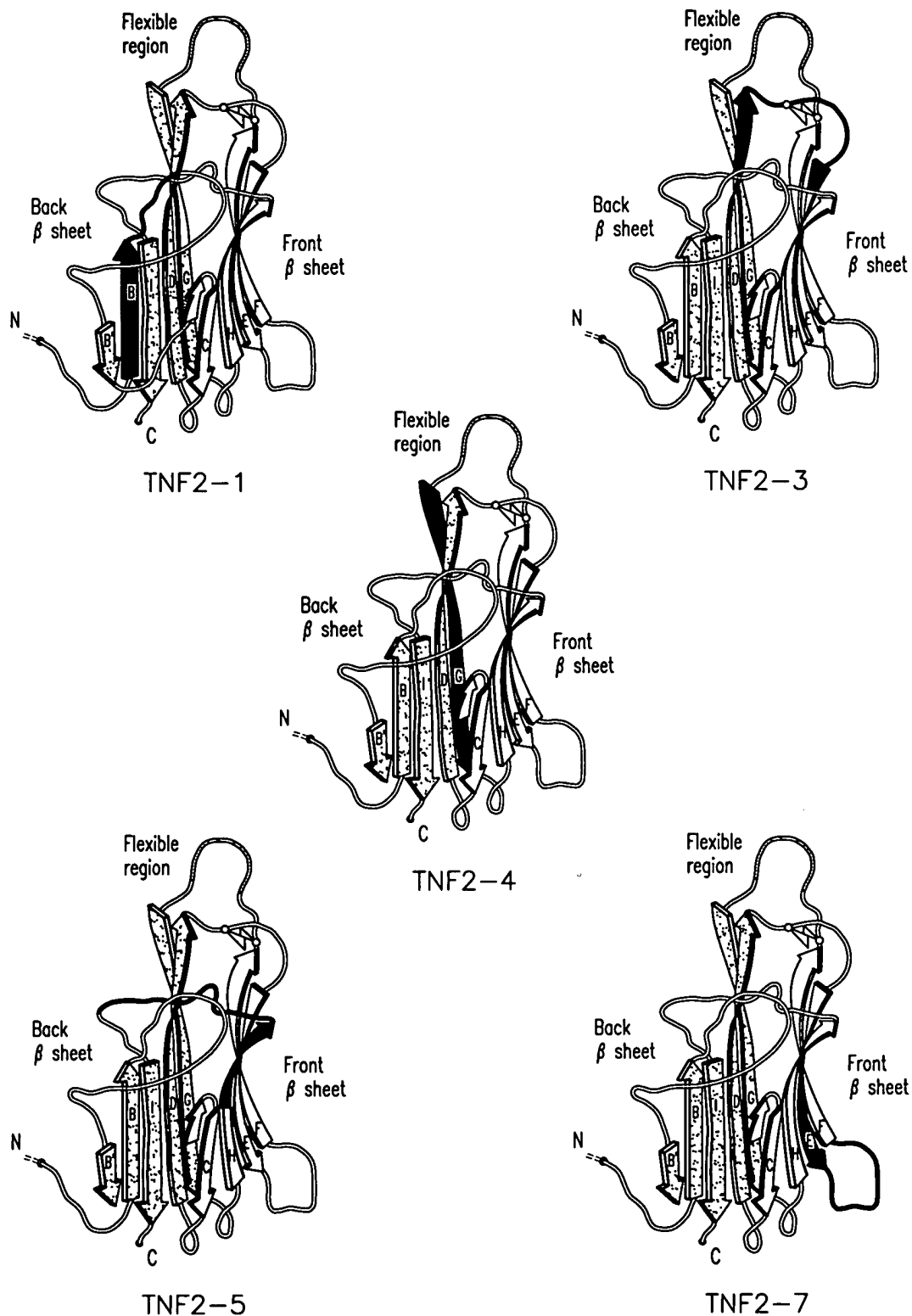




FIG. 5b

TNF α analogs with the P30 epitope inserted

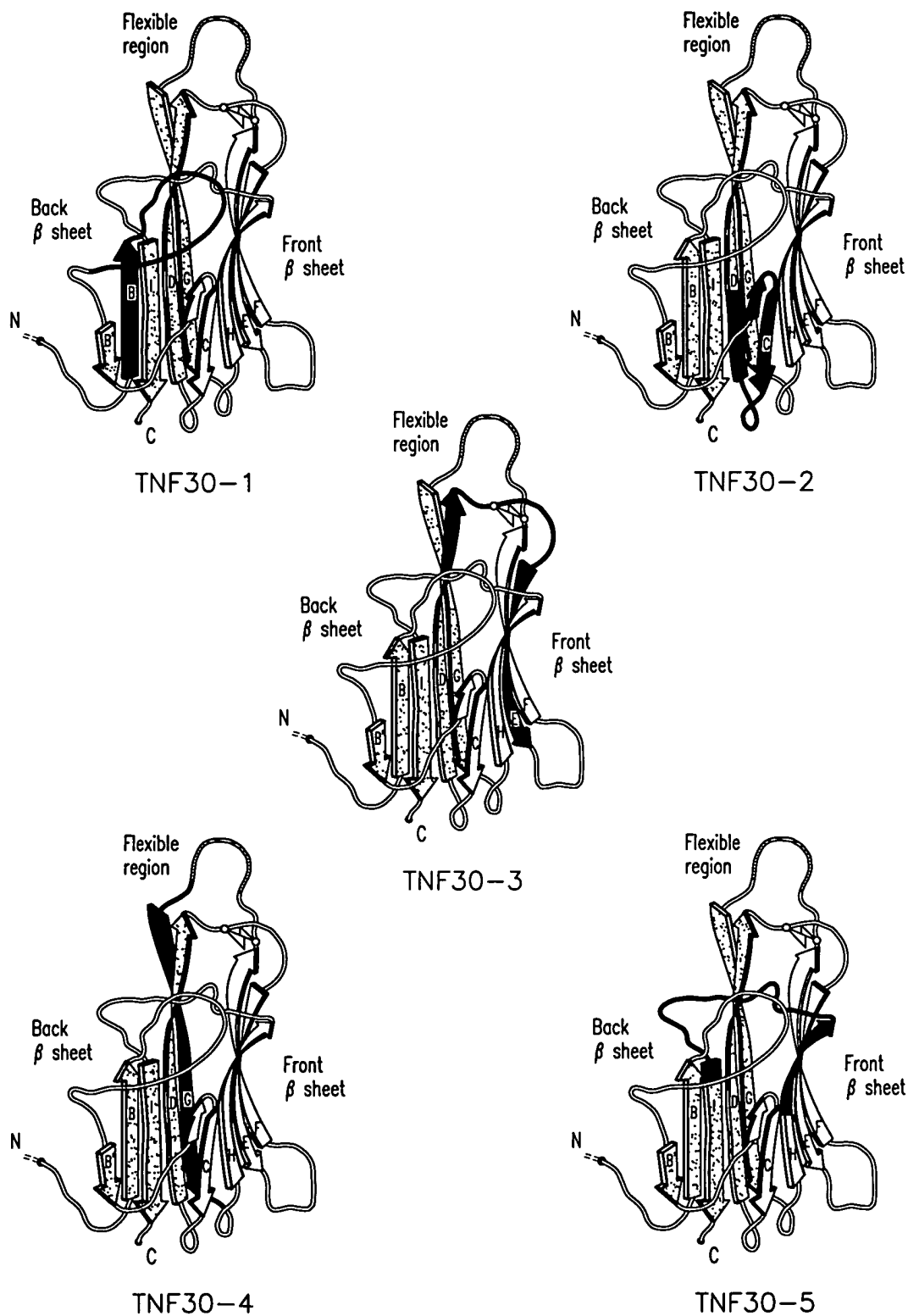


FIG. 6

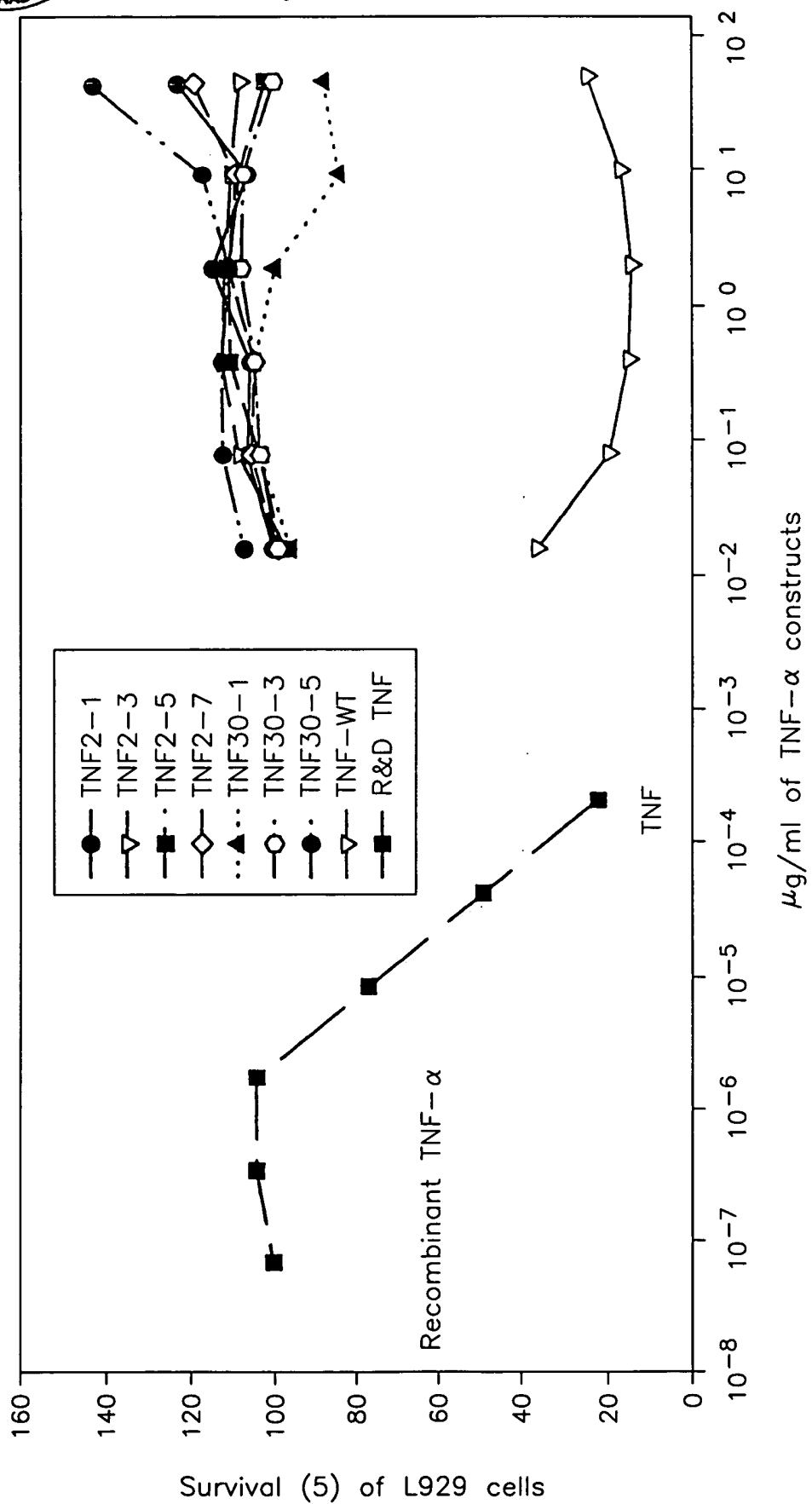
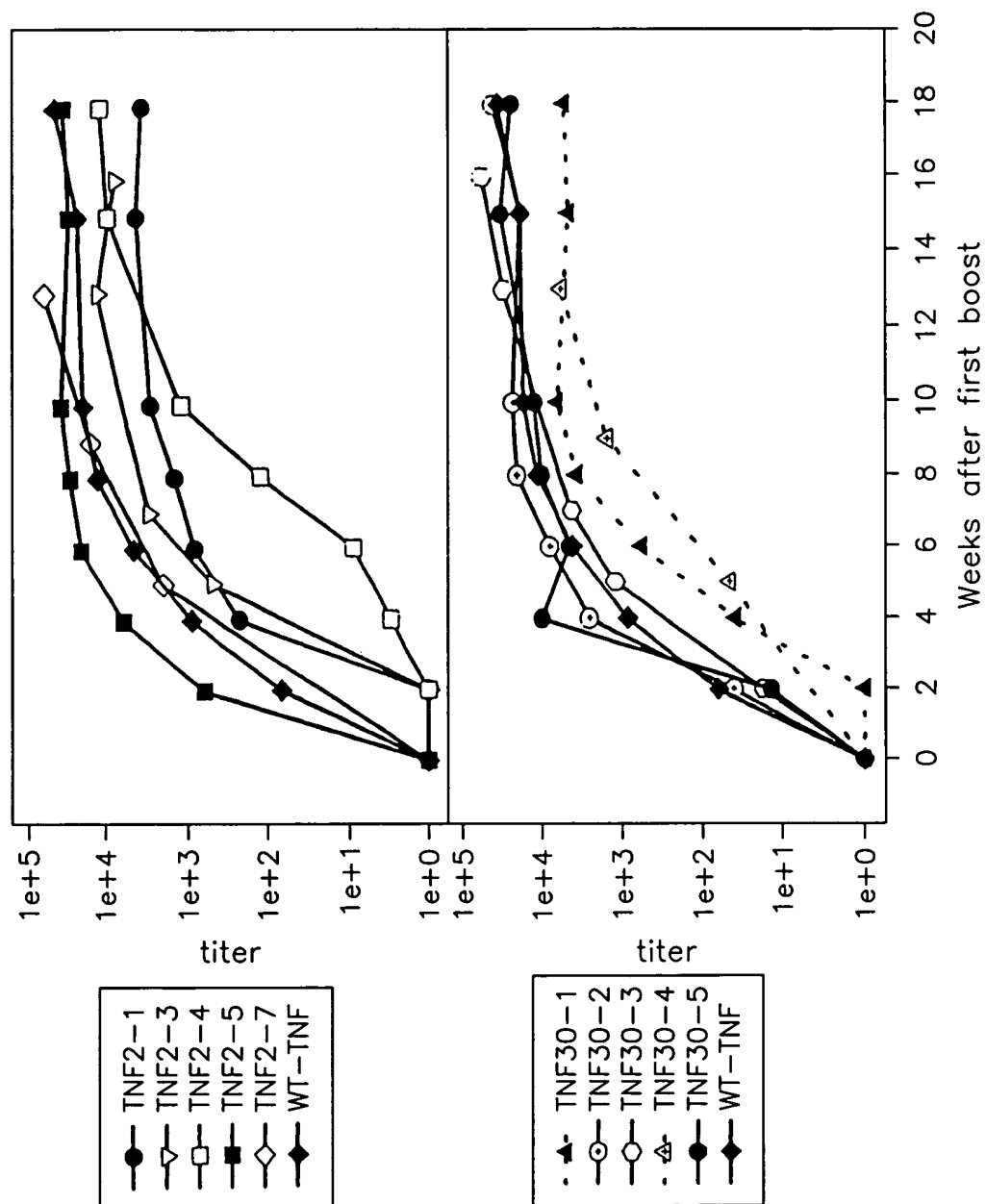


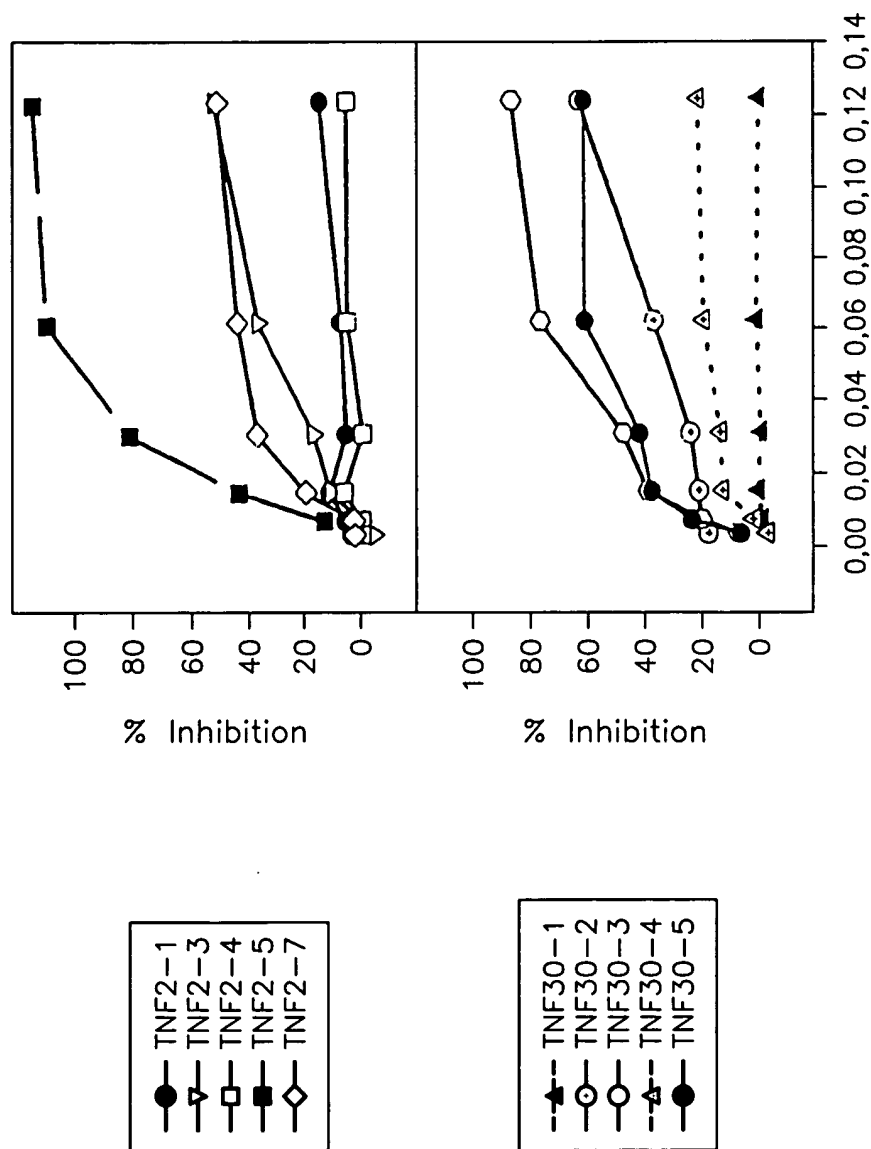


FIG. 7

The anti human TNF antibody response in rabbits



The ability of P2/P30 modified human TNF α molecules to induce neutralizing antibodies as measured in the L929 cell assay



The ability of P2/P30 modified human TNF α molecules to induce neutralizing antibodies as measured in the receptor assay

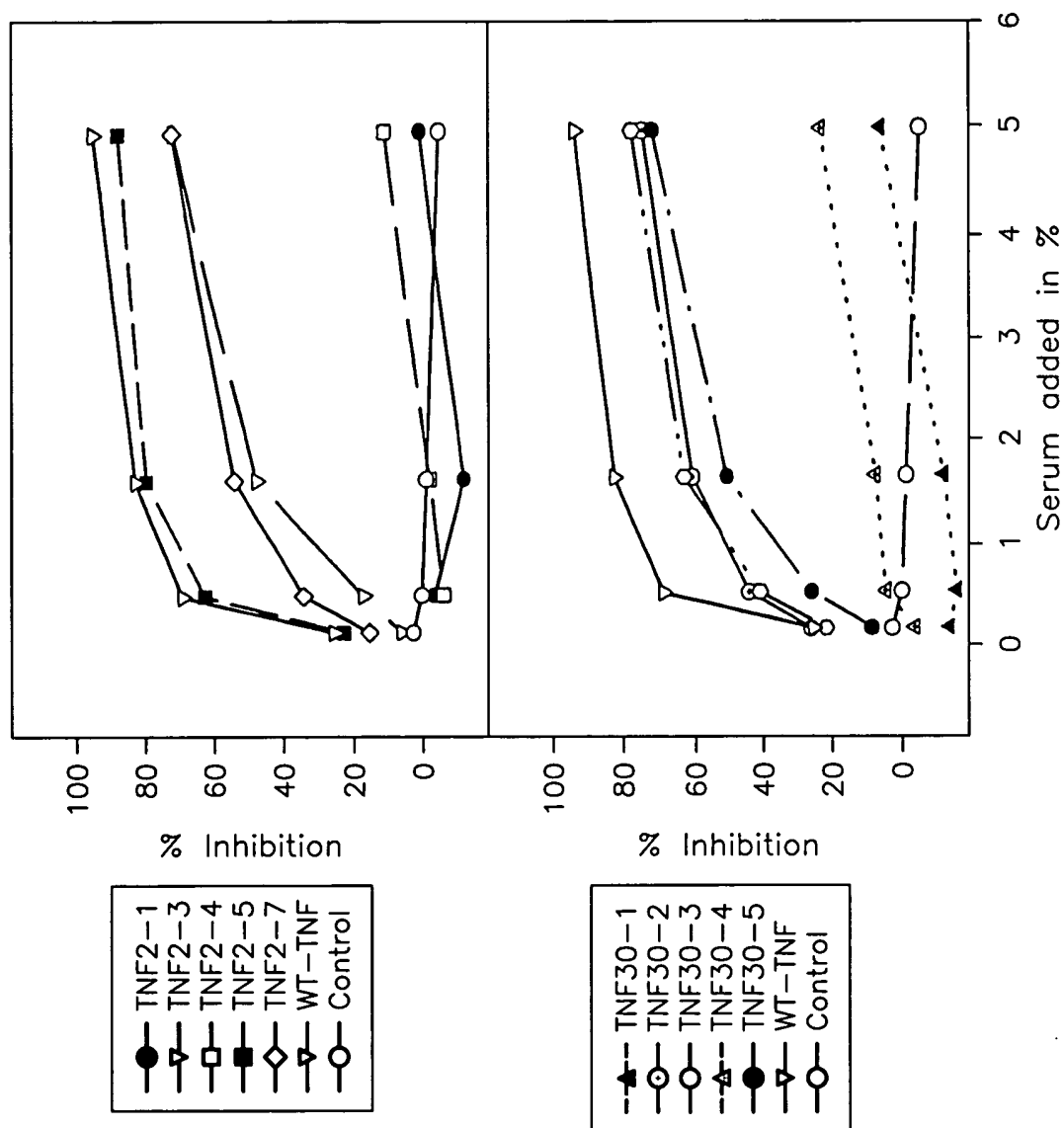


FIG. 10

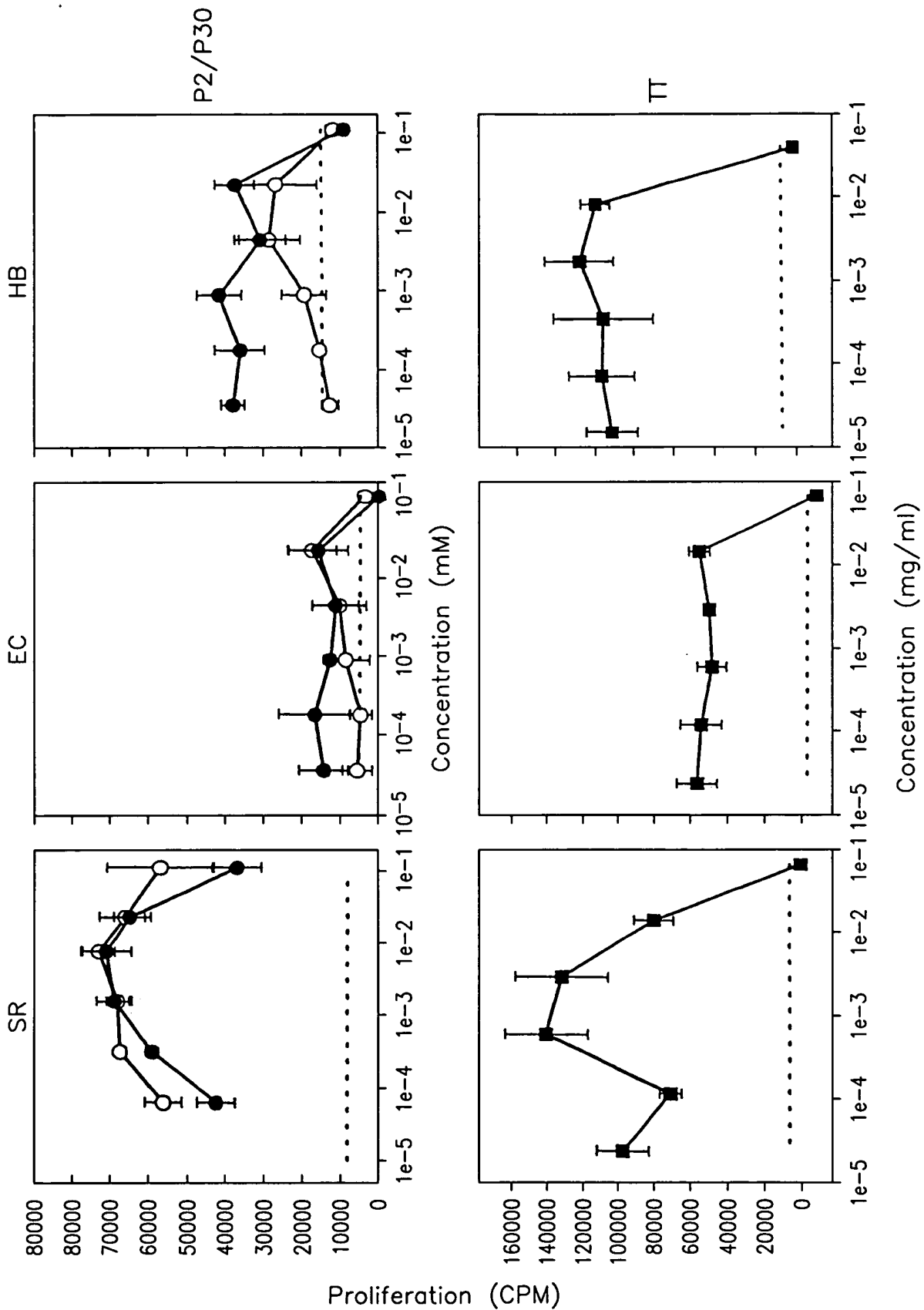


FIG. 11

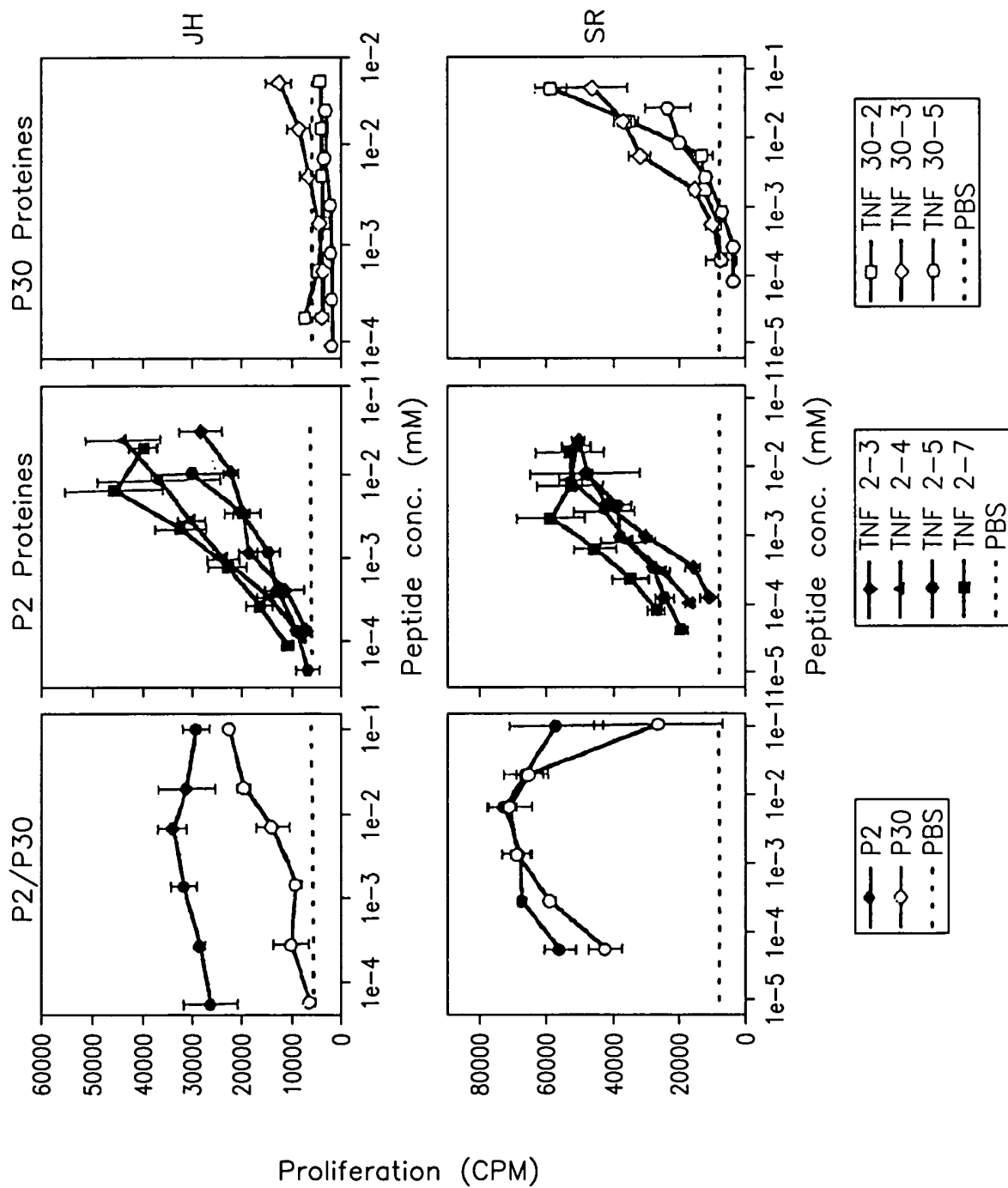




FIG. 12

PBMC Assays

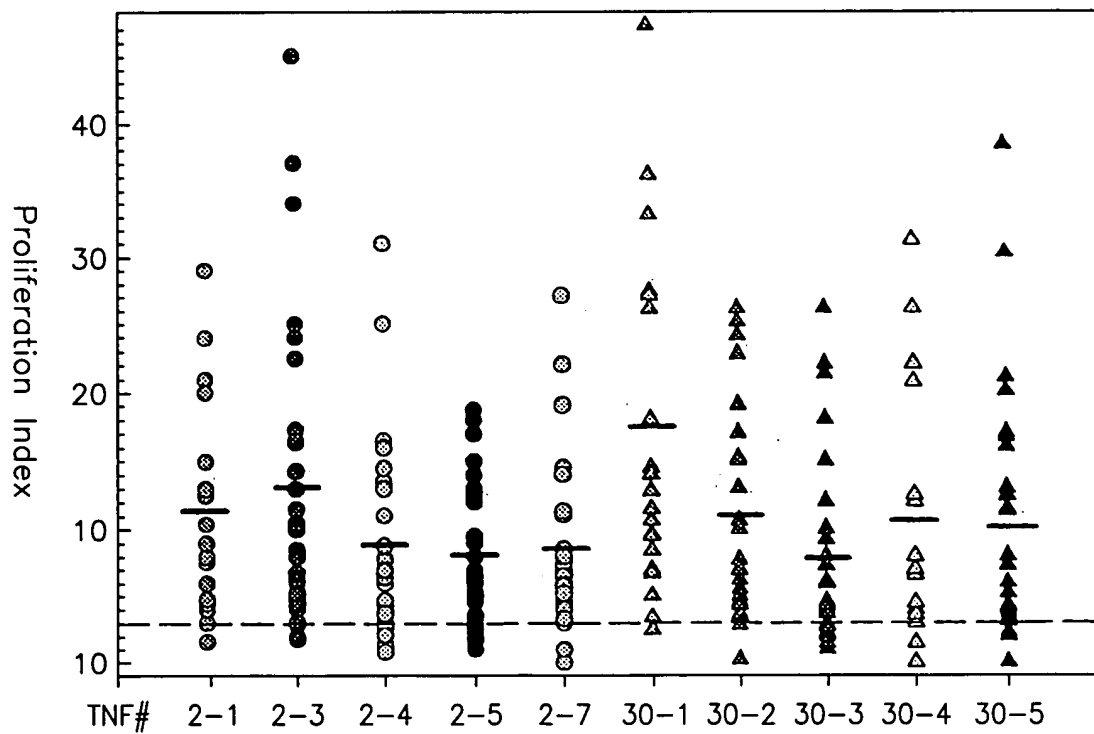




FIG. 13

The PBMC response against P2 and P30 modified TNF proteins in P2 and P30 specific responders, respectively

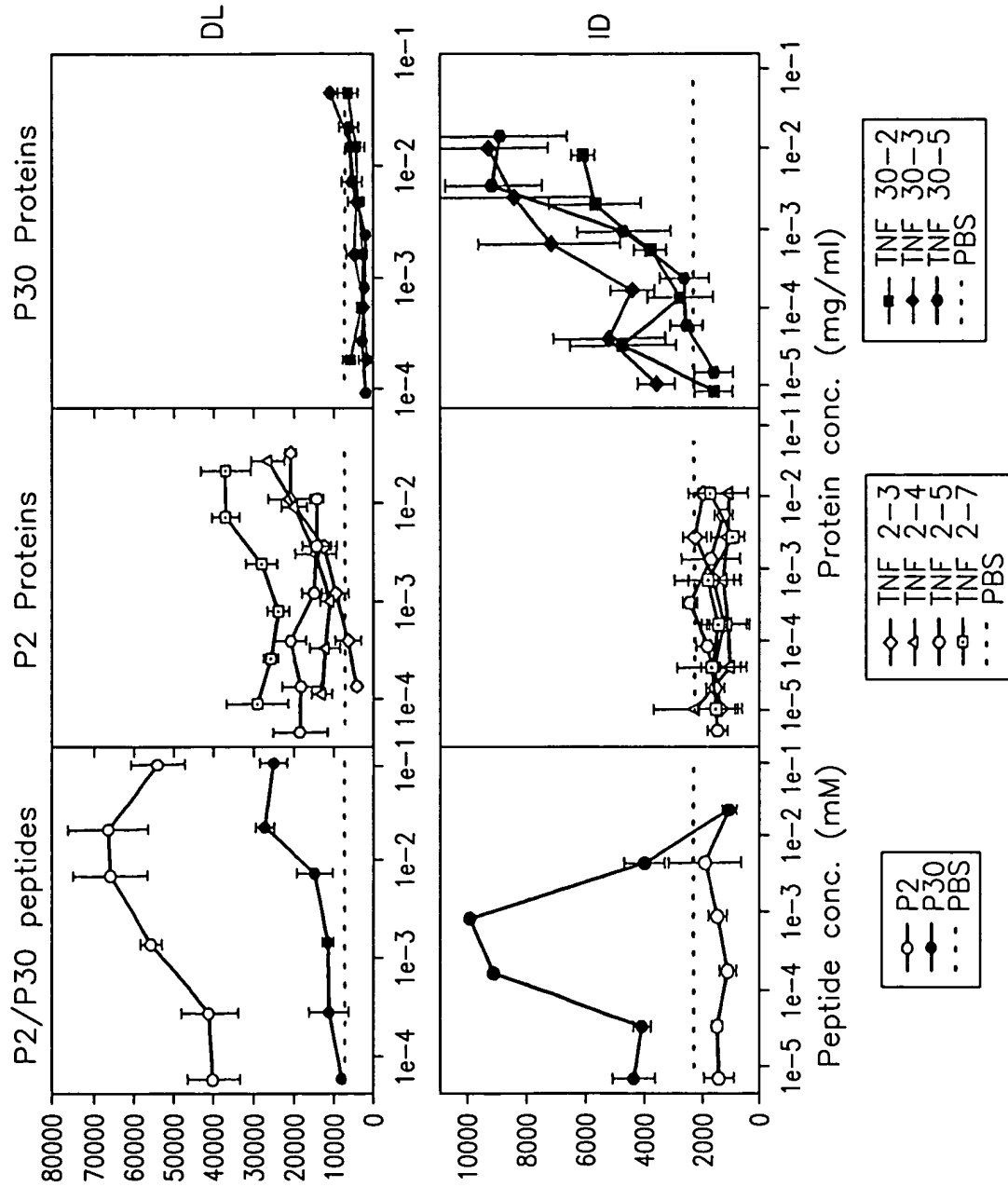


FIG. 14

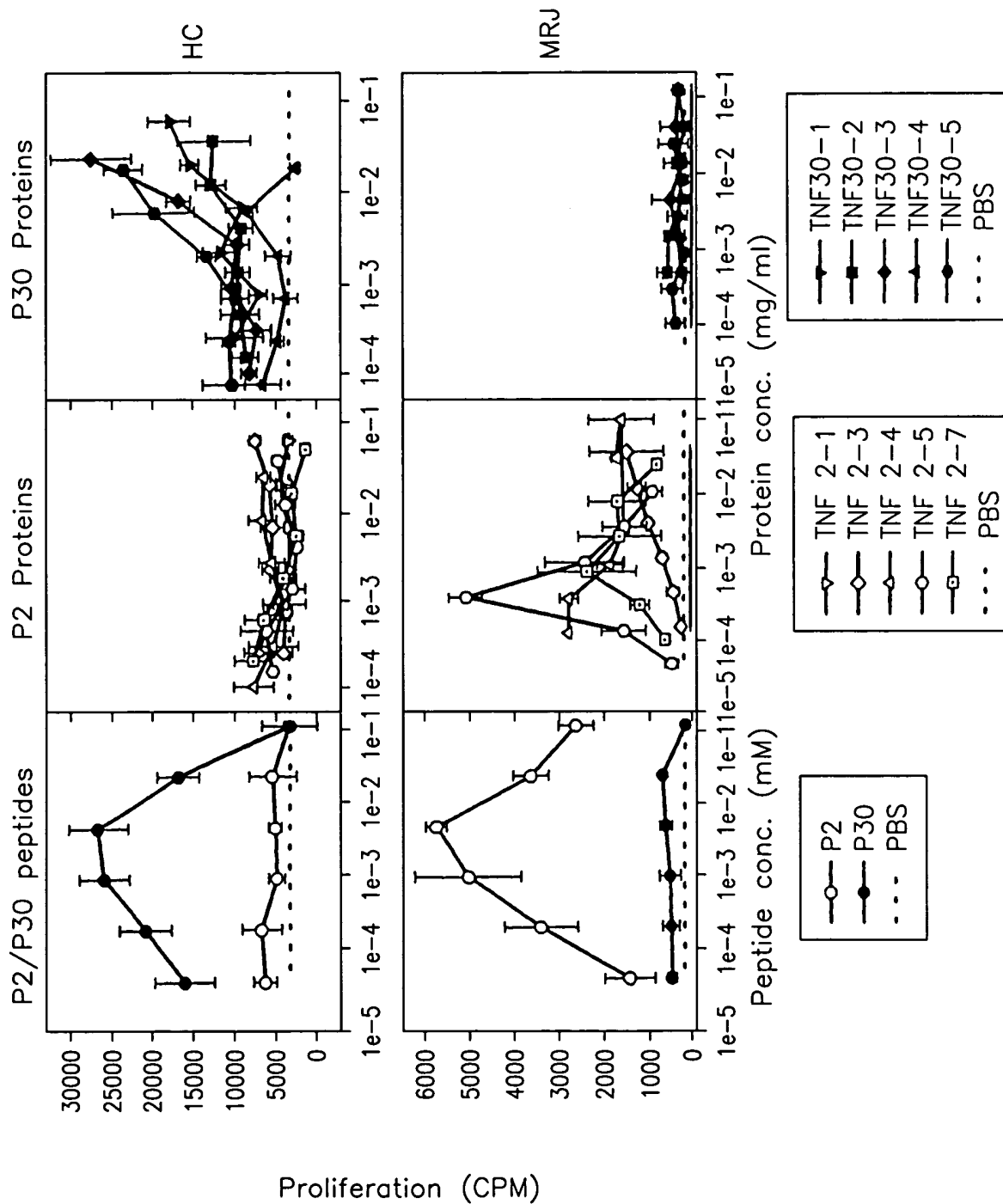




FIG. 15

The influence of flanking amino acids on the T cell recognition of P2 and P30

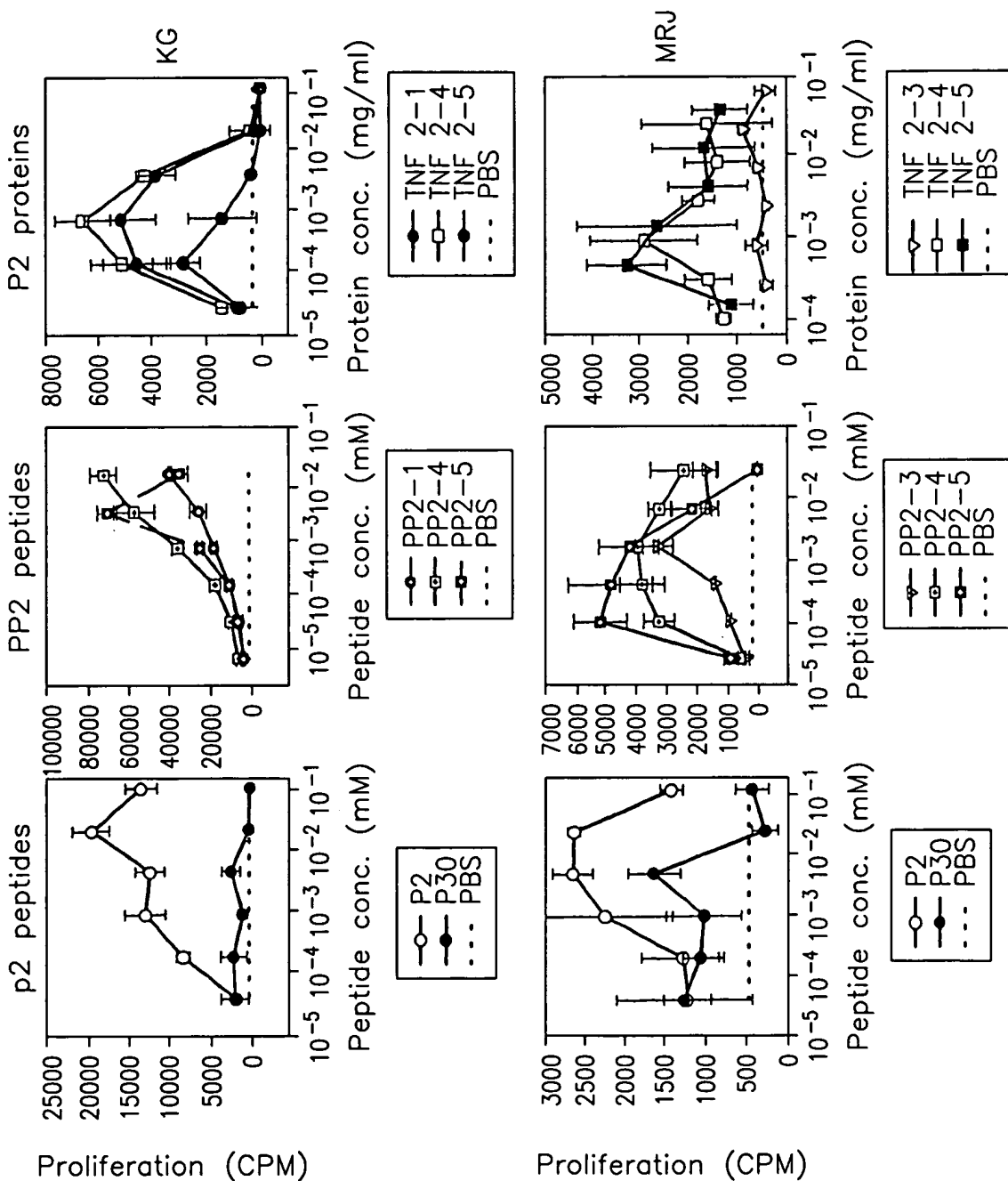




FIG. 16
Mutation Strategy

